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Range extension of the Atlantic Forest Hocicudo, *Oxymycterus dasytrichus* (Schinz, 1821), to the state of Santa Catarina, southern Brazil

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Abstract: Six individuals of *Oxymycterus dasytrichus* (Schinz, 1821) were found on the coast of Paraná and Santa Catarina (in the Atlantic Forest), expanding the known geographical distribution of the species ca. 280 km southward. The specimens represent the first record of the species for the state of Santa Catarina, and new localities to the region of southern Paraná. The identification of the species relied mainly on interspecies comparative assessment of genetic distance based on DNA sequences data from the mitochondrial cytochrome-*b* gene and geographic distribution of taxa across biomes, particularly in the Atlantic Forest. Our findings highlight the role of protected areas, particularly the Guaraqueçaba Environmental Protection Area, to preserve small mammals.

Key words: Rodentia; Akodontini; Dense Ombrophilous Forest

The Neotropical sigmodontine rodent genus *Oxymycterus* Waterhouse, 1837 is diverse, with 15 recognized species (Oliveira and Gonçalves 2015), and geographically widespread, from central Argentina and southern Uruguay to the Amazon basin (Hershkovitz 1994). The species boundaries, phylogenetic relationships, and distribution remain poorly understood (Jayat et al. 2008). Several recent studies have significantly advanced the understanding of the taxonomy and phylogeny of *Oxymycterus* (e.g., Hoffmann et al. 2002; Gonçalves and Oliveira 2004; D'Elía et al. 2008; Jayat et al. 2008; Oliveira and Gonçalves 2015). However, distinct aspects of the geographic distribution of the species are still in question, particularly in highly diverse biomes such as the Atlantic Forest.

Oxymycterus dasytrichus (Schinz 1821), commonly named the Atlantic Forest Hocicudo (Bonvicino et al. 2008), is widely distributed in Dense Ombrophilous Forest along the Brazilian coast, in the states of Pernambuco, Sergipe, Alagoas, Bahia, Espírito Santo, Minas Gerais, Goiás, Rio de Janeiro, and São Paulo, from the coastal lowlands to altitudes of around 2,000 m in Itatiaia, Rio de Janeiro (Oliveira 1998; Hoffmann et al. 2002; Gonçalves and Oliveira 2004; Oliveira and Gonçalves 2015). Oliveira and Gonçalves (2015) also recorded this species to the state of Paraná, although they do not list specific localities. The type locality was restricted to the Rio Mucuri in Bahia (Avila-Pires 1965). Gonçalves and Oliveira (2004) used integrative molecular and morphological techniques to study this species over most of its distribution in the Atlantic Forest, and their results suggested the existence of a morphological differentiation along a north–south axis.

The species is currently classified as of Least Concern (LC) according to the IUCN Red List of Threatened Species, due to its wide distribution and high population densities (Bonvicino et al. 2008).

The present study expanded the distributional range of *O. dasytrichus* (Figure 1) along the Atlantic Forest, to ca. 280 km south of the nearest previously recorded locality, at Cananéia, São Paulo. The four new localities are on the coast of Paraná (PR) and Santa Catarina (SC) (Figure 2; Table 1). The climate is humid subtropical (Köppen Cfa) (Kottek et al. 2006); the mean annual

Figure 1. *Oxymycterus dasytrichus*. Photograph by Pablo R. Gonçalves.

rainfall on the coast of Paraná is 2,435 mm, and on the north coast of Santa Catarina is 1,690 mm, with mean annual temperatures of 21 and 20.2°C, respectively (Vanhoni and Mendonça 2008; Araujo et al. 2006). The new recording localities are:

I) Guaraqueçaba-Índios, PR (25°21′ S, 048°26′ W, 10 m above sea level [a.s.l.]), on the northern coast of Paraná, flanked by Paranaguá and Laranjeiras bays and the Serra do Mar coastal mountain range. The Guaraqueçaba Environmental Protection Area (APA) includes the largest continuous remnant of the Atlantic Forest on the Brazilian coast. Together with Vale do Ribeira and

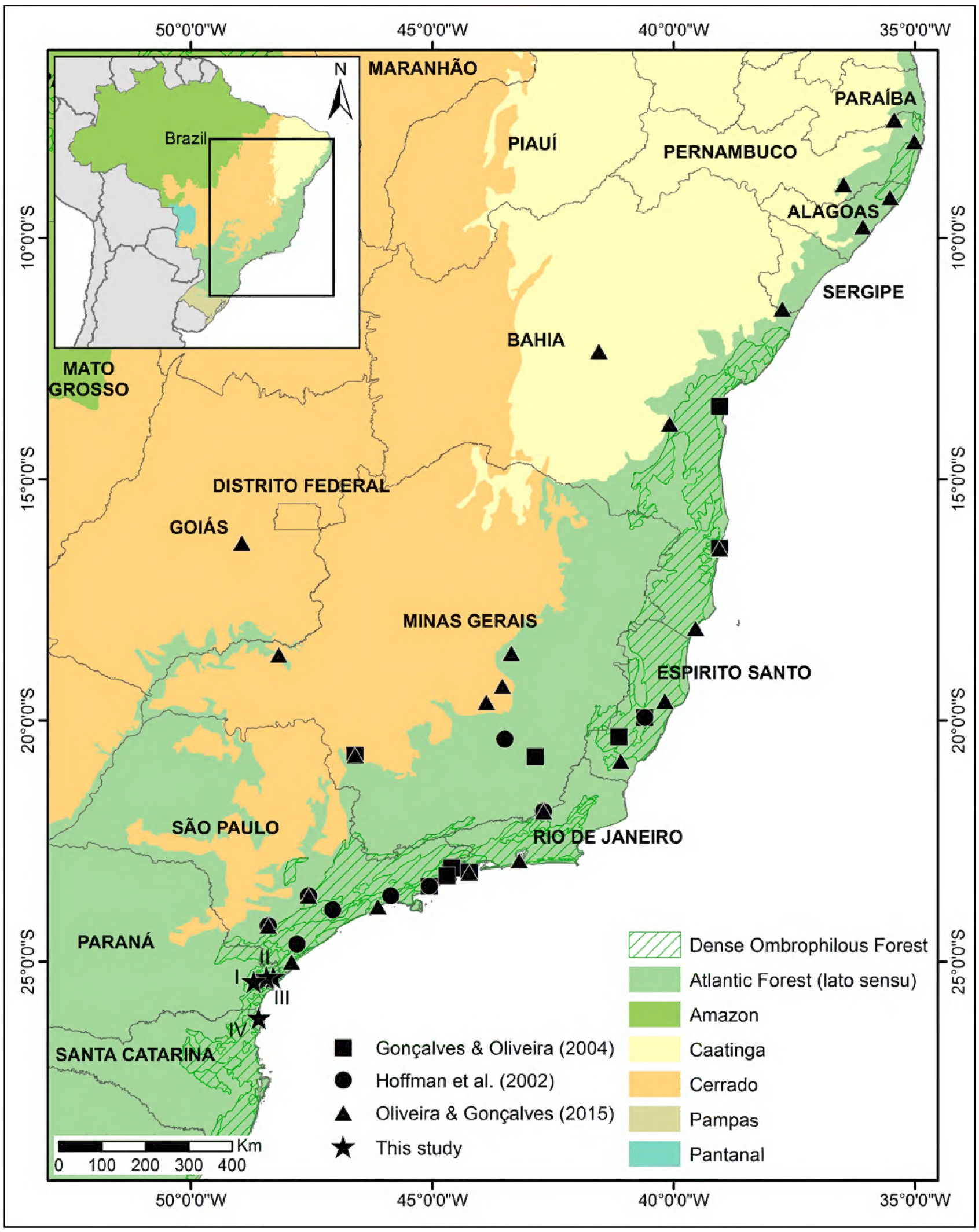

Figure 2. *Oxymycterus dasytrichus* distribution, primarily along the Atlantic Forest biome in Brazil. Stars indicate the locations of four new records from the Paraná and Santa Catarina coast. Numbers from I to IV are indicated in Materials and Methods.

Table 1. Locality records for *Oxymycterus dasytrichus*, including four new sites presented in this study.

State	Municipality	Locality	Latitude (S)	Longitude (W)	Reference*
Santa Catarina	Itapoá	—	26°10′	048°36′	This study
Paraná	Guaraqueçaba	Índios	25°21′	048°26′	This study
Paraná	Guaraqueçaba	Massarapuã	25°19″	048°26′	This study
Paraná	Antonina	Bacia do Rio do Nunes	25°25′	048°42′	This study
São Paulo	Juquitiba	Barra do Rio Juquía	23°55′	047°04′	1
São Paulo	Salesópolis	Estação Biológica de Boracéia	23°38′	045°52′	1
São Paulo	—	—	24°37′	047°48′	1
São Paulo	Salto de Pirapora	Salto de Pirapora	23°38′	047°34′	1
São Paulo	Ubatuba	Ubatuba	23°26′	045°04′	1
São Paulo	Cananéia	Barra do Guarú	25°00′	047°55′	3
São Paulo	Capão Bonito	Fazenda Intervales	24°15′	048°24′	3
São Paulo	Salto de Pirapora	Salto de Pirapora	23°38′	047°34′	3
São Paulo	Bertioga	Varjão	23°51′	046°08′	3
São Paulo	Capão Bonito	Fazenda Intervales	24°15′	048°24′	1
São Paulo	Ubatuba	Ubatuba	23°26′	045°04′	2
Rio de Janeiro	Angra dos Reis	Ilha Grande	23°09′	044°14′	2
Rio de Janeiro	Paraty	Tarituba	23°03′	044°36′	2
Rio de Janeiro	Paraty	Paraty	23°13′	044°42′	2
Rio de Janeiro	Angra dos Reis	Ilha Grande	23°09′	044°14′	3
Rio de Janeiro	Rio de Janeiro	Tijuca, Fazenda Velha	22°54′	043°12′	3
Espírito Santo	Santa Teresa	Santa Teresa	19°56′	040°36′	2
Espírito Santo	Venda Nova do Imigrante	Venda Nova	20°20′	041°08′	2
Espírito Santo	Santa Teresa	Santa Teresa	19°56′	040°36′	1
Espírito Santo	Cachoeiro de Itapemirim	Castelinho	20°50′	041°06′	3
Espírito Santo	Santa Teresa	Reserva Florestal Nova Lombardia (Augusto Ruschi)	19°35′	040°11′	3
Minas Gerais	Além Paraíba	Além Paraíba	21°53′	042°42′	1
Minas Gerais	Ouro Preto	Ouro Preto	20°23′	043°30′	1
Minas Gerais	Passos	Passos	20°43′	046°36′	2
Minas Gerais	Viçosa	Viçosa	20°45′	042°52′	2
Minas Gerais	Além Paraíba	Além Paraíba	21°53′	042°42′	3
Minas Gerais	Araguari	Rio Jordão	18°38′	048°11′	3
Minas Gerais	Lagoa Santa/Conceição do Mato Dentro	Boca da Mata, km 104–105	19°02′	043° 25′	3
Minas Gerais	Serro	Fazenda Esperança	18°36′	043°22′	3
Minas Gerais	Lagoa Santa	Lagoa Santa	19°37′	043°53′	3
Minas Gerais	Passos	Passos	20°43′	046°36′	3
Goiás	Anápolis	Anápolis	16°19′	048°57′	3
Bahia	Cairu	Fazenda Subauma	13°29′	039°03′	2
Bahia	Palmeiras (Chapada Diamantina)	Gerais da Cachoeira da Fumaça	12° 31′	041° 33′	3
Bahia	Porto Seguro	Reserva Biológica Pau Brasil	16°26′	039°03′	3
Bahia	Mucuri	Rio Mucuri	18° 05′	039° 33′	3
Bahia	Jequié	Três Braços	13°51′	040° 05′	3
Sergipe	Cristinápolis	Fazendo Cruzeiro	11°28′	037°45′	3
Alagoas	Matriz de Camaragibe	Fazenda Santa Justina	09°09′	035°31′	3
Alagoas	São Miguel dos Campos	Mangabeiras	09°46′	036°05′	3
Pernambuco	Macaparana	Fazenda Água Fria	07°33′	035°26′	3
Pernambuco	São Lourenço da Mata	São Lourenço da Mata	08°00′	035°01′	3
Pernambuco	Garanhuns	Garanhuns	08°53′	036°29′	3

*1, Hoffmann et al. (2002); 2, Gonçalves and Oliveira (2004); 3, Oliveira and Gonçalves (2015). Geographical coordinates from 2 and 3 were estimated based on the municipality and/or location available.

Serra da Graciosa, the Guaraqueçaba APA is part of the Biosphere Reserve. The area adjacent to this sample locality also covers the Guaraqueçaba Ecological Station, including Superagüi National Park, and the Salto Morato and Sebuí Private Natural Heritage Reserves. Two males (UFPR-P752, UFPR-P754) and one female (UFPR-P760) were collected.

II) Guaraqueçaba-Massarapuã, PR (25°19′ S, 048°26′ W, 10 m a.s.l.), on a peninsula in the Guaraqueçaba APA. One male (UFPR-P757).

III) Antonina, basin of the Rio Nunes, PR (25°25′ S, 048°42′ W, 5 m a.s.l.) situated at sea level adjacent to the Serra do Mar on the coast of Paraná. One female (UFPR-P177).

IV) Itapoá, SC (26°10′ S, 048°36′ W, 18 m a.s.l.), on the northern coast of Santa Catarina, also in Dense Ombrophilous Forest (Atlantic Forest). One male (FURB 18669).

Specimens collected on the PR coast were caught in a Tomahawk trap, baited with corn and pineapple, in two field trips, one in June 1986 (UFPR-P177) and the other in April 1993 (UFPR-P752, UFPR-P754, UFPR-P757 and UFPR-P760). The single specimen captured on the northern coast of SC (FURB 18669; Figure 3) was caught in a Sherman trap baited with corn, in September 2011. These specimens are deposited in scientific collections at the Universidade Federal do Paraná (UFPR) and the Universidade Regional de Blumenau (FURB). Individuals were measured, tissues (muscle, kidney, liver and heart) were preserved in 96% ethanol at –20°C, and the skull and skin were prepared.

Total genomic DNA was purified from tissue samples of the six *O. dasytrichus* specimens using a PureLink DNA Extraction Kit (Invitrogen) following the manufacturer's instructions. A partial sequence (801 base pairs, bp) of the cytochrome-*b* (cyt-*b*) gene was amplified by polymerase chain reaction (PCR) using ca. 50 ng of DNA per sample, with primers and conditions described by Smith and Patton (1993). The PCR products were purified using the enzymatic method (Exonuclease I and Thermosensitive Alkaline Phosphatase, Thermo Scientific) and sequenced on an ABI 3700 sequencer (Applied Biosystems). Sequence data obtained were deposited in GenBank under accession numbers KU161271–KU161276 (Table 2).

In order to identify the genealogical position of the newly sequenced haplotypes of *O. dasytrichus*, we incorporated 11 sequences from southern Brazil (considered here as a reference for the species) provided by Hoffmann et al. (2002) and Gonçalves and Oliveira (2004) (Table 2). Ten other representative species of the genus were incorporated in the analysis (obtained from GenBank; Table 2) to discuss relationships of the species within the genus, using taxa with occurrence in Brazilian biomes, particularly the Atlantic forms, based on Gonçalves and Oliveira (2004) phylogenetic hypothesis. All data were aligned in the program MUSCLE (http://www.ebi.ac.uk/tools/msa/muscle) and manually adjusted in the software MEGA 6 (Tamura et al. 2013). The best-fit model of nucleotide substitution was determined based on the Akaike Information Criterion. Bayesian inference was used to reconstruct the phylogenetic relationships using BEAST 2.02 (Drummond et al. 2012) under the HKY model with a proportion of invariant sites and a gamma distribution. All parameters were estimated from the data. The Markov chains were run for 10 million generations and repeated four times to test for Markov chain–Monte Carlo chain convergence, and priors exceeded 200 to ensure effective sample sizes (ESS). Burn-in was determined in Tracer v1.5 (Drummond and Rambaut, 2007) based on parameter trajectories, and 10% of the first trees were then removed in TreeAnnotator. The consensus tree was visualized and edited in FigTree 1.3.1 (Rambaut 2009). Nodes with posterior probability

Figure 3. Dorsal, ventral and lateral views of the skull of *Oxymycterus dasytrichus* (CZFURB 18669) collected at Itapoá, Santa Catarina, Brazil. Photograph by Willian T. Peçanha. Scale bar = 10 mm.

Table 2. Sequences of the mitochondrial cytochrome b gene of *Oxymycterus* species used in the phylogenetic analysis and genetic distance comparisons, obtained from GenBank (accession number), with reference.

Species	GenBank Accession No.	Museum/ Institution ID*	Reference
O. hiska			
	U03542	MVZ 171518	Smith and Patton (1993)
O. nasutus			
	DQ518258	GD 577	Jayat et al. (2008)
	EF661854	—	Montes et al. (2008)
	AF175286	MVZ 182701	Hoffmann et al. (2002)
	AF175287	CA 695	Hoffmann et al. (2002)
O. josei			
	AF175288	MVZ 183264	Hoffmann et al. (2002)
	AF175289	MVZ 183265	Hoffmann et al. (2002)
O. amazonicus			
	AF454765	MZUSP 21317	Hoffmann et al. (2002)
O. delator			
	AF454766	UMMZ 137077	Hoffmann et al. (2002)
	AF454767	LPC 481	Hoffmann et al. (2002)
	U03525	UMMZ 133939	Smith and Patton (1993)
	DQ518256	MNHNP 2914	Jayat et al. (2008)
	AY275125	UMMZ 175101	D'Elía (2003)
O. dasytrichus			
	KU161273	UFPR-P752	This study
	KU161274	UFPR-P754	This study
	KU161276	UFPR-P760	This study
	KU161275	UFPR-P757	This study
	KU161272	UFPR-P177	This study
	KU161271	CZFURB 18669	This study
	AF454768	MVZ 183125	Hoffmann et al. (2002)
	AF454769	MVZ 183126	Hoffmann et al. (2002)
	AF454770	MVZ-JLP 16283	Hoffmann et al. (2002)
	AF454771	MNR-ML 125	Hoffmann et al. (2002)
	AF516662	MN 62254	Gonçalves and Oliveira (2004)
	AF516659	MN 65534	Gonçalves and Oliveira (2004)
	AF516658	MZUFV 659	Gonçalves and Oliveira (2004)
	AF516657	MN 65536	Gonçalves and Oliveira (2004)
	AF516663	MN 62258	Gonçalves and Oliveira (2004)
	AF516664	MN 62259	Gonçalves and Oliveira (2004)
	AF516665	MN 62260	Gonçalves and Oliveira (2004)
O. quaestor			
	AF454772	LG 41	Hoffmann et al. (2002)
	AF516660	MN 65544	Gonçalves and Oliveira (2004)
	AF516661	MN 65543	Gonçalves and Oliveira (2004)
O. judex			
	AF454773	MVZ 183128	Hoffmann et al. (2002)
	AF454774	MVZ 183129	Hoffmann et al. (2002)
O. rufus			
	AF454775	TK 49118	Hoffmann et al. (2002)
	AF454776	TK 49119	Hoffmann et al. (2002)
	AF454777	BAL 000511	Hoffmann et al. (2002)
	AF516652	PRG 903	Gonçalves and Oliveira (2004)
	AF516651	PRG 1013	Gonçalves and Oliveira (2004)
	AF516653	MZUFV 713	Gonçalves and Oliveira (2004)
	AF516654	MN 65522	Gonçalves and Oliveira (2004)
	AF516655	MN 65538	Gonçalves and Oliveira (2004)
	AF516666	AC 05	Gonçalves and Oliveira (2004)
	AF516667	AC 30	Gonçalves and Oliveira (2004)
	AF516668	PY 01	Gonçalves and Oliveira (2004)
	AF516669	PY 02	Gonçalves and Oliveira (2004)
	AY275126	UP AC004	D'Elía (2003)
	AY275127	UP 033	D'Elía (2003)
O. paramensis			
	DQ518259	CML 7251	Jayat et al. (2008)
	DQ518260	CNP 852	Jayat et al. (2008)
	DQ518261	CNP 850	Jayat et al. (2008)
O. wayku			
	DQ518262	CML 7247	Jayat et al. (2008)

* ***Brazil:*** *MZUSP, Museu de Zoologia da Universidade de São Paulo, São Paulo; MNR, ML, Museu Nacional de Rio de Janeiro, LG 41; MN, PRG, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Museu de Zoologia "João Moojen de Oliveira"; MZUFV, Universidade Federal de Viçosa, Minas Gerais; LPC 458 at the Universidade Federal de Minas Gerais, Brazil.* ***Paraguay:*** *MNHNP, Museo Nacional de Historia Natural del Paraguay.* ***Uruguay:*** *GD, field number of Guillermo D'Elía (vouchers will be deposited at Museo Nacional de Historia Natural y Antropología, Montevideo); CA, Laboratorio de Evolución, Facultad de Ciencias, Montevideo.* ***Argentina.*** *CNP, UP, Colección de Mamíferos del Centro Nacional Patagónico Puerto Madryn; CML, Colección Mamiferos Lillo, Tucumán; MLP, AC, PY, Museo de La Plata, Buenos Aires; BAL 00-05-11 at the Museo de Historia Natural de la Plata, Berisso.* ***United States:*** *UMMZ, Museum of Zoology, University of Michigan, Ann Arbor; MVZ (JLP), Museum of Vertebrate Zoology, University of California, Berkeley (JLP, field numbers of The Museum); TK, Texas Tech University, Lubbock.*

(PP) ≥ 95% were considered to be supported (Alfaro et al. 2003). In order to characterize the genetic divergence between *O. dasytrichus* collected in PR and SC and those used as a reference (from southeast Brazil), pairwise genetic distances were calculated using the Kimura 2-parameters (K2P) model with 1000 replications bootstraps.

Taxonomy within *Oxymycterus* has been controversial because of difficulties in detecting discrete morphological and cytogenetic variation (Musser and Carleton 1993; Hershkovitz 1994; Oliveira 1998; Gonçalves and Oliveira 2004). Therefore, the identification of the six vouchers was based on some external morphology characters (such as coat color and skull) coupled with comparative assessment of DNA sequences (the mitochondrial gene cytochrome b) and distributional ranges.

Oxymycterus dasytrichus presents a dark-brown to paler brownish red without strong lining of back in the dorsum and dark-gray to paler, cinnamon or pinkish buff in the ventral (Oliveira and Gonçalves, 2015). Its skull is robust, proportionally larger in width dimensions of braincase, interorbital region and zygomatic plates, with longer molar tooth rows (mean 5.6 mm) comparably to other species of Atlantic Coast (Figure 4). Its variation in cranial measurements was reported by Gonçalves and Oliveira (2004). On the other hand, some species predominantly occurs in certain biomes; for example, the Atlantic Forest in the case of *O. dasytrichus*.

In the absence of discrete characters in *O. dasytrichus*, the identification of specimens relied mostly on lesser genetic distance associated to geographically distinct groups, using the nucleotide BLAST tool from the

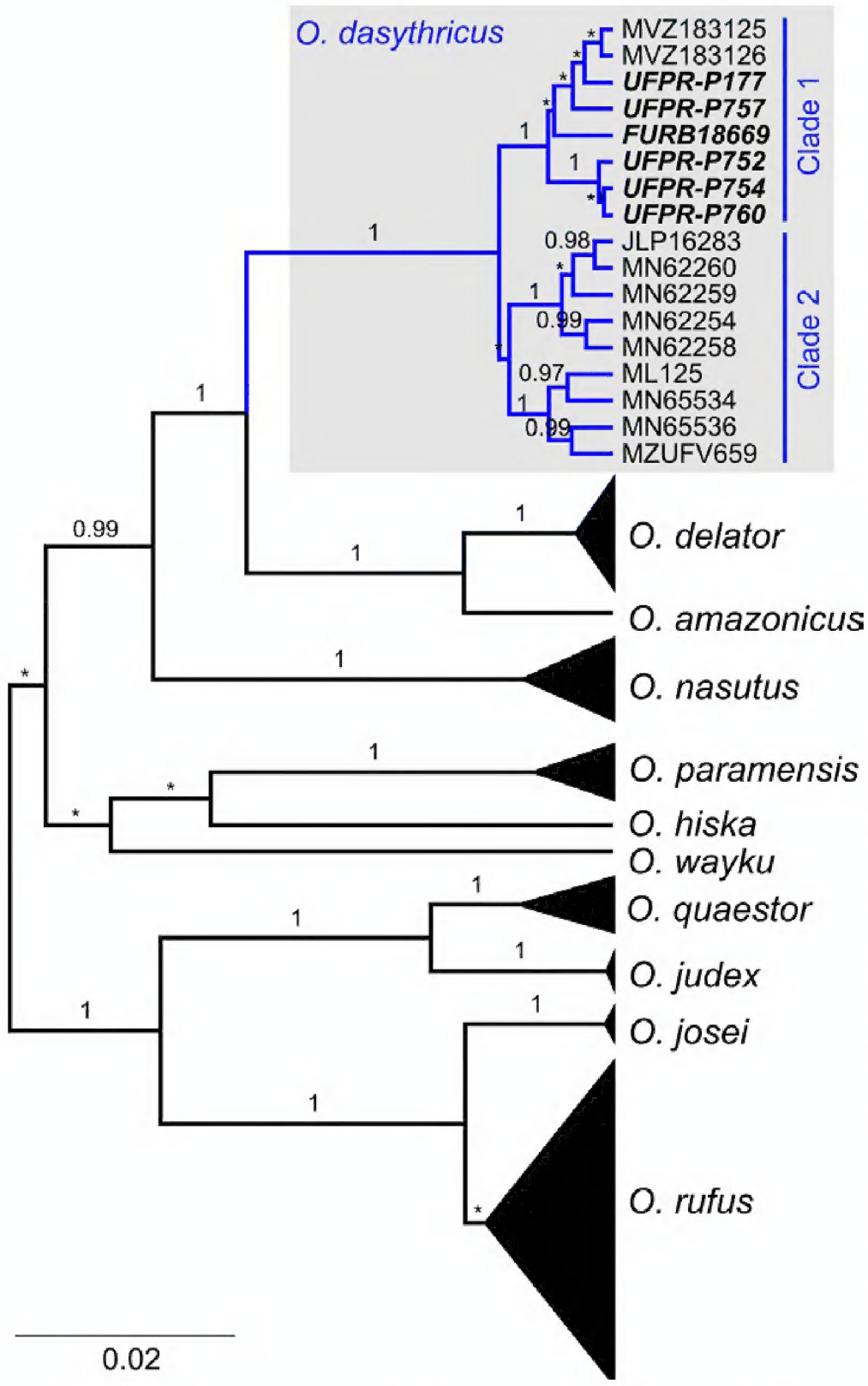

Figure 4. Bayesian consensus tree reconstructed based on 801 bp of the mitochondrial cytochrome b gene for *Oxymycterus dasytrichus* specimens collected in this study (UFPR-P752, UFPR-P754, UFPR-P760, UFPR-P757, UFPR-P177 and CZFURB 18669) and 11 sequences from the southeast, obtained from GenBank. Ten other lineages of *Oxymycterus* were incorporated (for specimen information see Table 2). Numbers above branches indicate posterior probability support. Asterisk represents values lower than 0.95.

NCBI database. Species presenting less than 5% of divergence collected in the same biome and assigned to *O. dasytrichus* by Gonçalves and Oliveira (2004), were used to attribute our samples to this taxon. Although some taxonomic mistakes are known to occur in the Genbank records, the consistency of close relationship to a large number of specimens collected along the Atlantic Forest previously identified as *O. dasytrichus* by experts in this group (using comparative morphology as well; Gonçalves and Oliveira 2004) led us to consider reliable such result.

The results from Bayesian inference analysis (Figure 4) indicated that the samples collected along the PR and SC coast belong to the *O. dasytrichus* group, because the haplotypes corresponding to the newly collected specimens from these sites are nested in the major clade formed by those previously referred as this species. Thus, our identification of the newly reported specimens relies on lesser divergence comparative to other species of the genus and diagnose made by Gonçalves and Oliveira (2004) of the specimens they reported from the Atlantic Forest, based on morphological and molecular data. Because no morphological diagnostic characters to identify species in this genus are known, in order to identify specimens we had to trust on comparisons based on genetic distances and morphology with previously identified specimens. Two internal haplogroups were recovered for *O. dasytrichus*: clade 1 formed by eight specimens/haplotypes (six from this study, and two from São Paulo and clade 2 formed by nine specimens/haplotypes from Rio de Janeiro, Espírito Santo, Minas Gerais and São Paulo. Whereas clade 1 received strong support, clade 2 was not supported. The K2P mean genetic distance between clades 1 and 2 was 2.1%, whereas that between species ranged from 6 to 12%.

Our data indicate that discoveries on general aspects of the biology (e.g., distributional range) of the genus *Oxymycterus* can still be made, even in a well-studied biome as the Atlantic Forest. Hoffmann et al. (2002) reported an intraspecific distance of 1.3% for the species across its northern and southern Atlantic Forest distribution. In our study we found similar divergence (1.4%), suggesting a shallow structure among haplotypes of clades 1 and 2. Such divergence does not seem to be determined strongly by a geographic cline since both clades include specimens from São Paulo. Thus, we did not find evidence to suggest that the populations represented by Clade 1 and Clade 2 are not conspecific. On the other hand, *O. dasytrichus* also occurs in the Caatinga and Cerrado (Costa and Leite 2012); therefore, such populations deserve particular effort in terms of comparative morphology and genetics since there is a greater distance to the Atlantic Forest populations and higher fixation of differences, and/or local adaptation to these distinct biomes, might exist.

The Atlantic Forest (lato sensu) coverage was reduced to 11–16% of the original area, in which 9% is protected areas, and 1% represents the original forest (Ribeiro et al. 2009). However, it is estimated that 1–8% of all the biodiversity in the planet is within this area (Silva and Casteleti 2003). According to Kauano et al (2012) the northern coast of Paraná is likely one of the least fragmented areas of the Atlantic, and is inserted in the "Serra do Mar", an important corridor of fauna and flora. Three of our four records were sampled in this region, which reinforces the significant role of protected areas, particularly the APA of Guaraqueçaba, not only to save small mammals, but as hotspot of endemic biodiversity refugee of the Atlantic Forest.

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